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Genetic and biologic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Colombia, South America

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Abstract

The prevalence of *Toxoplasma gondii* in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* in 77 free-range chickens (*Gallus domesticus*) from Colombia, South America was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT), and found in 32 (44.4%) of 72 chickens with titers of 1:5 in 4, 1:10 in 3, 1:20 in 1, 1:40 in 1, 1:80 in 8, 1:160 in 8, 1:320 in 3, and 1:640 or higher in 4. Hearts and brains of 31 seropositive chickens were pooled and bioassayed in mice. Tissues from 32 (16 + 16) seronegative chickens were pooled and fed to two, *T. gondii*-free cats, and tissues from nine chickens without matching sera were fed to one *T. gondii*-free cat. Feces of cats were examined for oocysts. *T. gondii* oocysts were excreted by a cat that was fed tissues of 16 seronegative chickens. *T. gondii* was isolated by bioassay in mice from 23 chickens with MAT titers of 1:20 or higher. All infected mice from 16 of the 23 isolates died of toxoplasmosis. Overall, 82 (81.1%) of 101 mice that became infected after inoculation with chicken tissues died of toxoplasmosis. Genotyping of these 24 isolates using polymorphisms at the SAG2 locus indicated that seven *T. gondii* isolates were Type I, 17 were Type III, and none was Type II. Phenotypically, *T. gondii* isolates from chickens from Colombia were similar to isolates from Brazil but different from the isolates from North America; most isolates from chickens from Brazil and Colombia were lethal for mice whereas isolates from North America did not kill inoculated mice. Genetically, none of the *T. gondii* isolates from Colombia and Brazil was SAG2 Type II, whereas most isolates from chickens from North America were Type II. This is the first report of genetic characterization of *T. gondii* isolates from Colombia, South America.
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Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Columbia; South America; Genotype

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1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or to other factors.

T. gondii isolates have been classified into three genetic types (I, II, III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997; Mondragon et al., 1998; Owen and Trees, 1999; Fuentes et al., 2001; Grigg et al., 2001; Ajzenberg et al., 2002, 2004; Boothroyd and Grigg, 2002; Jungersen et al., 2002; Aspinall et al., 2003; Dubey et al., 2004a,d; da Silva et al., 2005). The parasite was previously used to be considered clonal with very low genetic variability. However, most of the information was derived from isolates from Europe and North America. Using newer markers for genetic characterization and using recently isolated strains from Brazil and French Guiana, higher genetic variability was revealed than previously reported (Ajzenberg et al., 2004; Lehmann et al., 2004).

We have initiated a worldwide study of *T. gondii* population structure. For this we have chosen the free-range chicken as the indicator host for soil contamination with *T. gondii* oocysts because they feed from the ground. Thus far, we have characterized strains from South America (Brazil (Dubey et al., 2002, 2003a,d, in press-e), Peru (Dubey et al., 2004b), Venezuela (Dubey et al., in press-d), Argentina (Dubey et al., 2003e, in press-b)), Central America and the Caribbean (Guatemala (Dubey et al., in press-a), Grenada, West Indies (Dubey et al., 2005b)), North America (USA (Dubey et al., 2003c; Lehmann et al., 2003), Mexico (Dubey et al., 2004c)), Africa and Middle East (Egypt (Dubey et al., 2003b), Israel (Dubey et al., 2004e), Mali, Kenya, Burkina Faso, and Democratic Republic of Congo (Dubey et al., 2005a)), Asia (Sri Lanka (Dubey et al., in press-c), India (Sreekumar et al., 2003)), and Europe (Austria (Dubey et al., 2005c) and Portugal (Dubey et al., in press-g)). These studies are still not complete, nevertheless, a pattern is emerging that isolates from Brazil are genetically distinct (Lehmann et al., 2004).

In the present paper, we attempted to isolate and genotype *T. gondii* from chickens from Colombia, South America.

2. Materials and methods

2.1. Naturally-infected chickens

Chickens were obtained from free-range chickens in rural farms in Quindio region (center west of Colombia) 75°9′W 4°22′N, altitude 1483 m. Chickens ($n = 72$) were purchased during 12–16 April 2005. Chickens were collected, identified, and killed on one farm. Samples of brain, whole heart, and blood were collected from each chicken, and kept at 4 °C until sent with cold packs by air to Beltsville, MD. Three days elapsed between killing of chickens and receipt of samples at Beltsville. Samples were received in excellent condition.

2.2. Serological examination

Sera of chickens were tested for *T. gondii* antibodies using 4 dilutions, from 1:5 to 1:640 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

2.3. Bioassay of chickens for *T. gondii* infection

Tissues of all chickens were bioassayed for *T. gondii* infection. Brains, and hearts of 31 chickens with MAT titers of 1:5 or higher were bioassayed individually in outbred female Swiss Webster (SW) mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Tissues were homogenized, digested in acidic pepsin, washed, and homogenate inoculated subcutaneously into five mice (Dubey, 1998).

Brains and hearts from 32 (16 + 16) seronegative chickens were pooled and fed to two *T. gondii*-free cats (Dubey et al., 2002). Tissues from nine chickens without sera were fed to another cat. Feces of cats were examined for shedding of *T. gondii* oocysts 3–14 days post-ingesting chicken tissues as previously described (Dubey, 1995). Fecal floats were incubated in 2% sulfuric acid for one week at room temperature to allow sporulation of oocysts and were bioassayed orally in mice (Dubey and Beattie, 1988). Tissue imprints of lungs and brain of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 40–42 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 45–48 days p.i. and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

2.4. Genetic characterization for T. gondii

T. gondii DNA was extracted from the tissues of a single infected mouse from each group (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested PCR on the SAG2 locus according to Howe et al. (1997).

3. Results

Antibodies to *T. gondii* were found in 32 of 72 (44.4%) chickens with titers of 1:5 in 4, 1:10 in 3, 1:20 in 1, 1:40 in 1, 1:80 in 8, 1:160 in 8, 1:320 in 3, and 1:640 or higher in 4.

T. gondii was isolated from tissues of 23 chickens (Table 1); from 1 of 1 with a titer of 1:20, 1 of 1 with a titer of 1:40, from 7 of 8 with a titer of 1:80, and 14 of 15 with titers of 1:160 or higher. All infected mice from 16 of the 23 isolates died of toxoplasmosis. Overall, 82 (81.1%) of 101 mice that became infected after inoculation with chicken tissues died of toxoplasmosis. Most of the infected mice died of toxoplasmic pneumonia during the second and third week p.i. Twelve of the 23 isolates were from different locations.

Table 1
Isolation of *Toxoplasma gondii* from chickens from Colombia, South America

Chicken no.	Household designation, location	Chicken MAT titer	Isolation in mice[a] from chicken tissues			Isolate designation	Genotype
			No. infected[a]	No died	Day of death		
1	Salento, Cra 5	80	5	5	19, 19, 24, 24, 28	TgCkCo1	III
2	Salento, Cra 5	160	5	5	11, 17, 17, 17, 17	TgCkCo2	III
7	Salento, B Jardin	640	5	5	13, 14, 15, 15, 17	TgCkCo3	III
17	Salento, Criolinda	640	5	5	15, 15, 15, 17, 17	TgCkCo4	I
18	Salento, Criolinda	80	5	5	12, 15, 17, 17, 17	TgCkCo5	I
45	Salento, Alto Coronel	20	5	5	11, 13, 14, 15, 25	TgCkCo6	III
46	Montenegro Campo Alegre	40	5	5	15, 15, 19, 19, 36	TgCkCo7	III
47	Montenegro Campo Alegre	160	5	5	13, 14, 14, 17, 17	TgCkCo8	III
48	Montenegro Campo Alegre	80	4	0	n/a[b]	TgCkCo9	I
49	Circasia Llanitos	160	3	1	19	TgCkCo10	III
50	Circasia Llanitos	160	5	5	14, 14, 15, 15, 16	TgCkCo11	III
51	Circasia Llanitos	160	5	2	18, 19	TgCkCo12	III
52	Circasia Buenos Aires	80	1	0	n/a	TgCkCo13	III
59	Calarca Salida	80	5	5	12, 13, 14, 15, 15	TgCkCo14	I
62	Calarca Salida	160	5	5	13, 14, 15, 17, 17	TgCkCo15	III
65	Armenia Salvador Allende F. 1	640	5	5	13, 14, 15, 17, 17	TgCkCo16	III
66	Armenia Salvador Allende F. 1	80	5	3	20, 22, 24	TgCkCo17	I
67	Armenia Salvador Allende F. 1	160	5	5	11, 12, 13, 15, 18	TgCkCo18	III
70	Armenia Salvador Allende F. 2	320	3	3	14, 15, 16	TgCkCo19	III
71	Armenia Salvador Allende F. 2	640	5	0	n/a	TgCkCo20	I
72	Armenia Salvador Allende F. 2	320	2	0	n/a	TgCkCo21	III
75	Armenia Salvador Allende F. 3	160	5	5	13, 13, 14, 15, 15	TgCkCo22	I
77	Armenia Salvador Allende F. 4	80	3	3	16, 18, 21	TgCkCo23	III

[a] Five mice were inoculated with tissues of each chicken.
[b] not applicable.

One cat fed pools of tissues from 16 seronegative chickens shed oocysts; the mice inoculated with oocysts died of toxoplasmosis 5–6 days p.i. and the tachyzoites obtained from this isolate were lethal for mice. Thus, 17 of 24 isolates from asymptomatic chickens from Colombia were lethal for mice. The *T. gondii* isolates obtained by bioassay in mice were designated TgCkCo1–23 (Table 1). The isolate obtained by bioassay in cat was designated TgCkCo24.

Genotyping of these 24 isolates using polymorphisms at the SAG2 locus indicated that seven *T. gondii* isolates were Type I, 17 (16 isolates by bioassay in mice and one by bioassay in cat) were Type III, and none was Type II.

4. Discussion

In the present study *T. gondii* was isolated by bioassay in mice from 23 of 32 (71.8%) seropositive chickens and not from 8 chickens with titers of 1:5 and 1:10. Data from this and other studies with chickens (see Dubey et al., in press-b) are being accumulated for the validity of MAT for the detection of *T. gondii* in chickens. It is of interest that *T. gondii* was isolated from the feces of a cat that was fed tissues of 16 seronegative chickens but not from a cat that was fed pools of tissues from the other 16 seronegative chickens.

The success of isolation also depends on the number of mice inoculated, the amount of tissue bioassayed, and the concentration of the parasite in tissues sampled. In the present study, entire brains and hearts were used to isolate *T. gondii* and most of the tissue digest was inoculated into five mice. It is noteworthy that 103 of 115 (89.6%) mice inoculated with tissue digests from 23 infected chickens acquired toxoplasmosis, indicating that the concentration of *T. gondii* in tissues of chickens from Colombia was high. Pooling of brain and hearts for bioassay in mice might have contributed to the high recovery rate of the parasite.

Before the recognition of three genotypes of *T. gondii* (Howe and Sibley, 1995) *T. gondii* isolates were phenotypically classified as mouse virulent or avirulent. Type I strains were considered mouse virulent whereas Type II and Type III strains were avirulent or mildly virulent for mice (Howe and Sibley, 1995); Type I strains killed all mice within 2 week p.i., irrespective of the dose. However, these data are based on isolates that have been maintained in mice for an unknown time (Howe and Sibley, 1995). There are very few data on mouse mortality based on primary isolations. We have started to accumulate such data based on isolates from chickens using a specified protocol (subcutaneous inoculation of tissue digest into five SW mice). In the present study, 17 of the 24 isolates were lethal for mice, that is similar to the behavior of *T. gondii* isolates from Brazil but different from other countries. Most isolates from Brazil are mouse virulent, irrespective of the genotype, whereas strains from North America are generally avirulent for mice.

In the present study seven isolates were Type I and 17 isolates were Type III. The absence of Type II from Colombia and Brazil is remarkable. Although the data from Colombia are from a small sample, data from Brazil are based on 110 *T. gondii* isolates from chickens from geographically distinct regions (Dubey et al., 2002, 2003a,d, in press-e). However, the proportion of *T. gondii* types in Brazil and Colombia is different; 70% of isolates from chickens from Brazil were Type I and 30% were Type III, the reverse was for isolates from Colombia (29% Type I and 71% Type III).

Phenotypically and genetically, *T. gondii* isolates from chickens from Colombia were different from the isolates from North America; most isolates from chickens from Brazil and Colombia were lethal for mice whereas isolates from North America did not kill inoculated mice. Genetically, none of *T. gondii* isolates from Colombia and Brazil was SAG2 Type II, whereas most isolates from chickens from North America were Type II (Dubey et al., 2003c; Lehmann et al., 2003). This is the first report of genetic characterization of *T. gondii* isolates from Colombia, South America.

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